

# Seasonal Distribution, Biology, and Human Attraction Patterns of Culicine Mosquitoes (Diptera: Culicidae) in a Forest near Puerto Almendras, Iquitos, Peru

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**ABSTRACT** This study was conducted as part of a field ecology study of arboviral activity in the Amazon Basin, Peru, to determine the taxonomy, frequency, seasonal, and vertical distributions of potential mosquito vectors. In addition, the relative efficiency of human-landing collections and dry ice-baited Centers for Disease Control (CDC)-type light traps was determined for collecting mosquitoes. A total of 70 species of mosquitoes from 14 genera were collected from June 1996 through December 1997 at a forested site near Puerto Almendras, ≈20 km west-southwest of Iquitos, Peru. Three species [*Psorophora (Janthinosa) albigena* (Perry), *Ochlerotatus (Ochlerotatus) fulvus* (Wiedemann), and *Ochlerotatus (Ochlerotatus) serratus* (Theobald)] accounted for 70% of all mosquitoes captured in human-landing collections. Overall, biting activity occurred throughout the 24-h cycle but was higher during the daytime, primarily because of large populations of two day-biting species, *Ps. albigena* and *Oc. serratus*. *Oc. fulvus* was active throughout the 24-h cycle but was more frequently collected during the evening. *Oc. fulvus*, *Ps. albigena*, *Culex (Melanoconion) pedroi* Sirivanakarn & Belkin, and a mixture of *Culex (Melanoconion) vomerifer* Komp, and *Culex (Melanoconion) gnomatos* Sallum, Huchings & Ferreira, accounted for 73% of the mosquitoes captured during darkness by human collectors. In general, *Ochlerotatus* spp. and *Psorophora* spp. were more commonly captured in human-landing collections, whereas most *Culex* spp. were more frequently collected in the dry ice-baited CDC-type light traps. In general, mosquito populations were lowest from June through August when river levels were at their lowest. Two large population peaks occurred in November–December and in February–March as a result of “flood water” mosquito populations (e.g., *Ps. albigena*). These data provide a better understanding of the taxonomy, population density, and seasonal distribution of potential mosquito vectors within the Amazon Basin region and allow for the development of appropriate vector and disease prevention strategies.

**KEY WORDS** Culicidae, distribution, mosquito ecology, Amazon Basin, Peru

WORLDWIDE, MOSQUITOES ARE IMPLICATED as vectors of numerous arboviruses and other parasitic disease

pathogens affecting human and animal health. In the Amazon Basin, the distribution and epidemiology of arboviruses are inadequately described; this emphasizes the need for taxonomic and ecological studies of hematophagous Diptera as potential vectors of arboviruses (Momen 1998).

The emergence and expansion of zoonotic diseases are particularly sensitive to ecological changes, population movements, and the intrusion of humans and domestic animals into sylvatic environments. Ecological factors (e.g., deforestation, agricultural development, colonization, and urbanization of the Amazon

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Basin) have increasingly placed human and domestic animal populations at risk to malaria, leishmaniasis, and arboviral diseases (Marques 1979). For example, Mayaro and Oropouche virus infections were prevalent among Brazilian woodcutters who cleared the Amazonian forest in recent years (Murphy 1998). Ecotourism and expanding human settlements in the rainforests of the Amazon Basin are becoming more popular, increasing human risk to various known and yet undescribed arboviruses and other pathogens transmitted by biting flies. Additionally, human febrile illnesses of unknown origin, some of which are now being identified based on infections in mosquito populations, are common in the Amazon Basin in and near Iquitos, Peru (Watts et al. 1998; Tesh et al. 1999; D.M.W., unpublished data).

The bionomics of mosquito populations and the epidemiology of mosquito-borne diseases in the Amazon Basin are poorly understood, and the impact that these vector populations and disease pathogens that they transmit have on local and immigrant populations and agricultural, forestry, and military operations is largely unstudied. Although a few of the potential vectors of various arboviruses have been identified, vector distribution, epidemiology of associated diseases, and risk factors associated with the transmission of these viruses are unknown throughout most of the Amazon Basin.

However, with the introduction of humans, domestic animals, and various human activities that increase exposure to vector populations, an understanding of the biology and ecology of potential mosquito vectors is crucial for disease-threat analysis and for the development and implementation of vector-disease control strategies. Therefore, to acquire a better understanding of the risk of hematophagous arthropod-transmitted pathogens, we concurrently used human-landing collections (HLCs) and Centers for Disease Control (CDC)-type light traps (LTs) (American Biophysics Corp., East Greenwich, RI) baited with dry ice to collect mosquitoes. These data were used to determine the population density, daily activity rhythms, vertical distributions, seasonal distributions, and arboviral infection rates (reported separately) of mosquito species in a forested area near Iquitos, Peru. This study was part of a vector ecology-arboviral research program designed to evaluate the threat that arboviral diseases pose to human and animal populations in this region and is the most comprehensive vector bionomics study conducted in the Amazon Basin to date. The Puerto Almendras area was selected because human cases of dengue, Mayaro, Oropouche, Venezuelan equine encephalomyelitis (VEE), and large numbers of fevers of unknown origin were previously reported in nearby Iquitos (Chavez et al. 1992; Phillips et al. 1992; Hayes et al. 1996; Watts et al. 1997, 1998; Tesh et al. 1999).

#### Materials and Methods

**Study Site.** The study site ( $03^{\circ} 49' 34''$  S,  $73^{\circ} 22' 21''$  W) is located in a forested area  $\approx 400$  m northeast of

a rural village, Puerto Almendras,  $\approx 20$  km west-southwest of Iquitos. The site is bordered on one side by the Nanay River, a tributary of the Amazon River, and otherwise surrounded by forest and open farmland. The area had been deforested and a reforestation project of native trees was initiated in the late 1950s. Thus, the forest is composed of both primary and secondary growth trees, the oldest estimated to be  $\approx 45$  yr old, and is currently managed as the Arboretum "El Huayo," as part of the Botanic Garden at the Centro de Investigación y Enseñanza Forestal Puerto Almendra of the Faculty of Forestry Engineering, Universidad Nacional de la Amazonía Peruana. This study area is located at an altitude of 115 m and consists of numerous deciduous trees, including *Cedrelina catanaeformis* Durke, *Caryocar glabrum* (Aubl.), *Bertolletia excelsa* H.B.K., and *Parckia* spp. with a canopy that extended  $\approx 20$ –30 m. In addition, there were numerous bromeliaceae and orchidaceae. Undergrowth is moderate due to canopy shade. Iquitos, Province of Maynas, Department of Loreto, Peru, is the nearest city, with an approximate population of 300,000, located in northeastern Peru.

The rural village of Puerto Almendras consists of single-dwelling homes and is located  $\approx 0.4$  km from the forest study site. Dogs and chickens were the most common peridomestic animals observed in the village. Feral animals [i.e., squirrel monkeys (*Saimiri* spp.), Woolly monkeys (*Lagothrix* spp.), bandicoots (*Nassua* spp.), macaws (*Ara* spp.), and parrots (*Amazona* spp.)] were kept as pets by some of the local residents. The local population includes hunters and gatherers, who have virtually hunted out most of the wild game and large rodents. Animals in forested areas surrounding Puerto Almendras include rodents (*Proechimys* spp., *Oryzomys* spp., and *Neacomys* spp.), marsupials (*Philander* spp., *Metachirus* spp., and *Marmosops* spp.), and sloths (*Choloepus hoffmanni* Peters and *Bradypus* spp.) (A. Gozalo, personal communication). Adjacent to the forest and  $<1$  km from the collection site, was open farmland with free-ranging water buffalo, cattle, and horses. Numerous birds, including parrots, were observed in the forest.

**Mosquito Collections.** A total of 10 adult mosquito collections were made at  $\approx 6$ -wk intervals from September 1996 through October 1997. Each collection consisted of four to six 24-h collections (two 12-h intervals) over a 10- to 16-d period. Volunteers who were involved in the HLC wore hooded, screened jackets to prevent mosquitoes and other biting flies from feeding on the upper parts of the body. Volunteers exposed the lower half of their legs and used aspirators to collect mosquitoes as they landed in an attempt to feed. Mosquitoes were collected for 40-min periods starting on the hour, followed by a 20-min rest break, from 0600 to 1740 hours (daytime collection period) and from 1800 to 0540 hours (nighttime collection period). Two collection teams of two collectors each were positioned 20 m apart in a forested area  $\approx 150$  m from a secondary dirt road approaching Puerto Almendras from Iquitos. One collector from each team was located near the base of a large tree,

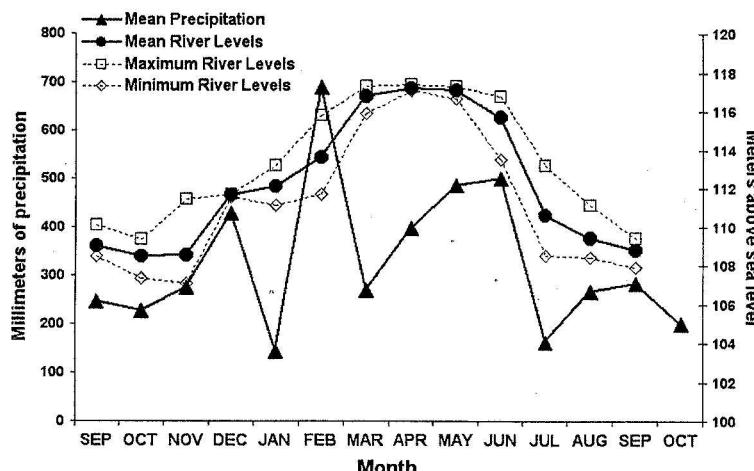


Fig. 1. Precipitation and river levels for Puerto Almendras from September 1996 to October 1997.

whereas the other collector was located on a platform in the canopy,  $\approx 10$  m above the forest floor. A palm frond roof was placed  $\approx 1.5$  m above the platforms in the canopy to protect the collector from weather (e.g., light rain). During each 40-min collection period, mosquitoes were aspirated from the collectors' exposed legs and placed in labeled, screen-topped, 0.5-liter cartons. At the end of each 40-min collection period, mosquitoes were provided water (saturated cotton) and placed in a styrofoam cooler. To reduce collector bias, the human collectors were rotated at the various collection locations on a daily basis. Concurrently, with the HLC, adult mosquitoes were collected using four dry ice-baited LTs (one pair of LTs was located  $\approx 15$  m from each of the two HLC teams). The traps were operated for 12-h periods from 0600 to 1800 hours and 1800 to 0600 hours. One LT from each pair was positioned 1.5 m above ground level, whereas the other one was positioned  $\approx 10$  m above the forest floor. Mosquitoes from the LT collections were placed in humidified coolers, provided water, and subsequently transported, along with the mosquitoes from the HLC, at the end of each 12-h collection period to a central laboratory in Iquitos where they were immobilized by chilling and identified over wet ice. After identification, the mosquitoes were pooled (25–50 specimens) according to species, placed in 1.5-ml cryovials, and then stored at  $-70^{\circ}\text{C}$  until assayed for virus at U.S. Army Medical Research Institute of Infectious Diseases (reported elsewhere).

**Mosquito Identification.** The identification of mosquitoes used in this study follow that of Lane (1953), Pratt (1953), Guedes and Souza (1964), Bram (1967), Pecor et al. (1992), and Sallum and Forattini (1996). These keys were not always appropriate because the taxonomy of many of the culicine mosquitoes in the Amazon Basin is poorly understood and is complicated by outdated keys and recent discoveries of species complexes. Therefore, representatives of field-collected mosquitoes were preserved, including progeny with associated larval and pupal exuviae for taxonomic

studies and fourth instars in absolute ethanol for DNA analysis. This material was deposited in the Walter Reed Biosystematics Unit, Smithsonian Institution, Washington, DC, where our field identifications were confirmed.

**Precipitation.** Minimum, maximum, and mean rainfall was provided by the Universidad Nacional Amazonas de Peruano (Fig. 1). Mean monthly Amazon River levels at Iquitos and total precipitation in Iquitos during September 1996 through October 1997 were calculated from daily data recorded by the Servicio de Hidrografía y Navegación de la Amazonía, Peruvian Navy, Iquitos, Peru, at a location  $\approx 15$  km from the study site.

## Results

**Mosquito Identification.** A diversity of mosquito species was collected in the rainforest near Puerto Almendras. Seventy species of mosquitoes assigned to 14 genera, including 24 species previously unreported from Peru (Pecor et al. 2000), were collected in HLCs or in dry ice-baited LTs (Table 1). Four species [*Psorophora albigena* (Peryassu), *Ochlerotatus fulvus* (Wiedemann), *Ochlerotatus serratus* (Theobald), and *Culex (Melanoconion) pedroi* Sirivanakarn & Belkin] accounted for 64% of the adult mosquitoes collected by all methods combined (Table 2). In addition, *Culex* (*Culex*) spp. (consisting almost entirely of *Culex coronator* Dyar & Knab, *Culex declarator* Dyar & Knab, and *Culex mollis* Dyar & Knab) accounted for an additional 8% of the mosquitoes.

Three species (*Ps. albigena*, *Oc. fulvus*, and *Oc. serratus*) accounted for 70% of all of the mosquitoes collected in the HLC (Table 3). Due to unresolved taxonomic status, many members of *Culex*, subgenera *Melanoconion* and *Culex*, were only identified to subgenus or species groups, and therefore are reported here as *Cx. (Mel.)* spp. and *Cx. (Culex.)* spp., respectively. Similarly, some members of the genera *Ochlerotatus*, *Sabethes*, *Uranotaenia*, and *Wyeomyia* were only identified to genera. Mosquitoes originally iden-

Table 1. Mosquito species captured in a forested habitat near Puerto Almendras, Peru

<i>Aedeomyia</i> ( <i>Aedeomyia</i> )	<i>janthinomys</i> Dyar
<i>squamipennis</i> (Lynch Arribalzaga)	
<i>Anopheles</i> ( <i>Anopheles</i> )	<i>Johnbelkinia</i>
<i>forattinii</i> Wilkerson & Sallum	<i>longipes</i> (F.)
<i>kompi</i> Edwards	<i>Limatus</i>
<i>mattogrossensis</i> Lutz & Neiva	<i>durhamii</i> Theobald
<i>peryassutii</i> Dyar & Knab	<i>flavisetosus</i> de Oliveira-Castro
<i>shannoni</i> Davis	<i>Mansonia</i> ( <i>Mansonia</i> )
<i>Anopheles</i> ( <i>Nyssorhynchus</i> )	<i>amazonensis</i> (Theobald)
<i>benarrochi</i> Gabaldon, Cova Garcia & Lopez	<i>flaveola</i> (Coquillett)
<i>darlingi</i> Root	<i>humeralis</i> Dyar & Knab
<i>numeztovari</i> Gabaldon	<i>indubitans</i> Dyar & Shannon
<i>oswaldoi</i> (Peryassu)	<i>pseudotitillans</i> (Theobald)
<i>triannulatus</i> (Neiva & Pinto)	<i>titillans</i> (Walker)
<i>Coquillettidia</i> ( <i>Rhynchotaenia</i> )	<i>Ochlerotatus</i> ( <i>Ochlerotatus</i> )
<i>albicosta</i> (Peryassu)	<i>fulvus</i> (Wiedemann)
<i>arribalzague</i> (Theobald)	<i>hastatus</i> (Dyar)
<i>hermanoi</i> (Lane & Coutinho)	<i>hortator</i> (Dyar)
<i>juxtamansonia</i> (Chagas)	<i>serratus</i> (Theobald)
<i>lynchi</i> (Shannon)	Undetermined <sup>a</sup>
<i>nigricans</i> (Coquillett)	<i>Ochlerotatus</i> ( <i>Protomacleaya</i> )
<i>venezuelensis</i> (Theobald)	<i>argyrothorax</i> Bonne-Wepster & Bonne
<i>Culex</i> ( <i>Carrollia</i> ) spp.	<i>Psorophora</i> ( <i>Grahamia</i> )
<i>Culex</i> ( <i>Culex</i> )	<i>cingulata</i> (F.)
<i>coronator</i> Dyar & Knab	<i>Psorophora</i> ( <i>Janthinosa</i> )
<i>declarator</i> Dyar & Knab	<i>albigenu</i> (Peryassu)
<i>mollis</i> Dyar & Knab	<i>ferox</i> (von Humboldt)
<i>Culex</i> ( <i>Melanoconion</i> )	<i>Psorophora</i> ( <i>Psorophora</i> )
<i>adamesi</i> Sirivanakarn & Galindo	<i>ciliipes</i> (F.)
<i>dunni</i> Dyar	<i>Sabettus</i> ( <i>Sabettus</i> )
<i>gnomatos</i> Sallum, Hutchings & Ferreira	<i>amazonicus</i> Gorden & Evans
<i>ocossa</i> Dyar & Knab	<i>cyanurus</i> (F.)
<i>olimpioi</i> Xavier, Da Silva & Da Silva Mattos	<i>quasicyaneus</i> Peryassu
<i>pedroi</i> Sirivanakarn & Belkin	<i>Sabettus</i> ( <i>Sabettoides</i> )
<i>portesi</i> Senevet & Abonnenc	<i>chloropterus</i> (von Humboldt)
<i>spissipes</i> (Theobald)	<i>Trichoprosopon</i>
<i>theobaldi</i> (Lutz)	<i>digitatum</i> (Rondani)
<i>vomerifer</i> Komp	<i>Uranotaenia</i> ( <i>Uranotaenia</i> )
<i>Atratus</i> Group	<i>apicalis</i> Theobald
Undetermined <sup>b</sup>	<i>geometrica</i> Theobald
<i>Culex</i> ( <i>Phenacomyia</i> )	<i>hystera</i> Dyar & Knab
<i>corniger</i> Theobald	<i>pallidoventer</i> Theobald
<i>Haemagogus</i> ( <i>Haemagogus</i> )	<i>pulcherrima</i> Lynch Arribalzaga
<i>baresi</i> Cerqueira	<i>Wyeomyia</i>
	Undetermined <sup>c</sup>

<sup>a</sup> At least one additional species of *Ochlerotatus* (*Och.*) was captured. See Pecor et al. (2000).<sup>b</sup> At least three distinct additional species of *Culex* (*Mel.*) were captured (J.E.P., unpublished data).<sup>c</sup> At least three distinct species of *Wyeomyia* were captured. See Pecor et al. (2000).

tified as *Culex* (*Melanoconion*) *vomerifer* Komp were subsequently described as two species, *Cx.* (*Mel.*) *vomerifer* and *Culex* (*Melanoconion*) *gnomatos* Sallum, Hutchings & Ferreira. These two species were identified by us, but are referred to as *Cx.* (*Mel.*) *vomerifer*/*gnomatos* throughout the rest of this article. Voucher specimens, supplemented by larval collections and selected rearing of progeny broods, allowed for the later specific identifications.

**Seasonal Distribution.** Seasonal distributions of selected culicine mosquitoes attracted to humans (daily means per collector for each collection period) are shown in Fig. 2. In general, total mosquito populations increased during the typical rainy season (September–May) and then slowly declined throughout the typical dry season (June–September). Adult mosquito populations were lowest for most species from June through August when river levels were at their lowest (Figs. 1 and 2a). Two large population peaks (November and February–March) of *Ps. albigena* and *Oc.*

*fulvus* accounted for >300 and >500 (November–December) and >2,000 and >500 (February–March) specimens per collector per day, respectively (Fig. 2a). Although present at lower levels, the seasonal abundance of *Ps. ferox* was similar to that of *Ps. albigena* and *Oc. fulvus* (Fig. 2b). *Oc. serratus* populations peaked in November 1996 during the beginning of the wet season, declined steadily during the remainder of the wet season, and then peaked again in August during the beginning or middle of the dry season (Fig. 2b). Several *Cx.* (*Mel.*) spp. (*Cx. pedroi*, *Cx. vomerifer*/*gnomatos*, and *Culex ocossa* Dyar & Knab) populations were very low from February to July but increased again during the later part of the dry season (July–August) when the river levels were declining (Fig. 2b and c). In contrast, populations of *Mansonia indubitans* Dyar & Shannon were greatest during the dry season (Fig. 2c).

**Nocturnal, Diurnal, and Daily Distribution.** Overall, mosquitoes were collected in HLCs throughout

**Table 2.** Mosquito species captured in human-landing collections and in dry ice-baited CDC-type light traps from September 1996 through October 1997 ( $n = 200$  trap-days) in a forested habitat near Puerto Almendras, Peru

Species	Mean no. captured/24 h <sup>a</sup>	% of Total <sup>b</sup>	Humans % <sup>c</sup>	Ground % <sup>d</sup>	Night % <sup>e</sup>
<i>Ps. (Jan.) albigena</i>	159.4	27.8	57	63	12
<i>Cx. (Mel.) spp. (total)<sup>f</sup></i>	141.5	24.6	25	61	94
<i>Oc. (Och.) fulvus</i>	126.4	22.0	48	48	77
<i>Cx. (Mel.) spp.<sup>g</sup></i>	53.5	9.3	15	54	86
<i>Cx. (Mel.) pedrooi</i>	47.2	8.2	24	55	99
<i>Cx. (Cux.) spp.<sup>h</sup></i>	45.8	8.0	11	53	99
<i>Oc. (Och.) serratus</i>	34.3	6.0	77	84	38
<i>Cx. (Mel.) vomerifer/gnomatos</i>	27.0	4.7	45	84	98
<i>Ps. (Jan.) ferox</i>	15.4	2.7	75	84	15
<i>Li. (Linn.) flavisetosus</i>	6.7	1.2	66	90	<1
<i>Ma. (Man.) indubitanus</i>	5.3	0.9	88	32	81
<i>An. spp.<sup>i</sup></i>	4.6	0.8	27	37	86
<i>Cx. (Mel.) theobaldi</i>	4.5	0.8	2	59	96
<i>Cq. (Rjm.) venezuelensis</i>	4.3	0.7	59	49	94
<i>Cx. (Mel.) ocoosa</i>	4.3	0.7	59	78	96
<i>Cx. (Mel.) spissipes</i>	4.3	0.7	18	75	99
<i>Oc. (Och.) hastatus</i>	3.0	0.5	41	73	13
<i>Li. (Linn.) durhamii</i>	2.4	0.4	33	87	<1
<i>Cx. (Mel.) Atratus Group</i>	1.8	0.3	15	60	94
<i>Hg. (Hag.) janthinomys</i>	1.7	0.3	97	29	<1
<i>Ps. (Gra.) cingulata</i>	1.6	0.3	55	50	95
OTHER (36 species)	22.9	4.0	31	52	57
All mosquitoes combined	574.0	100	44	60	59

<sup>a</sup> Mean number of mosquitoes captured per 24-h period (mean number of mosquitoes collected by all trapping methods).

<sup>b</sup> Percentage of all mosquitoes collected, regardless of trapping method or location.

<sup>c</sup> Percentage of that species collected by human collectors (number collected by human collectors at forest floor and in the canopy divided by total of that species collected by all methods).

<sup>d</sup> Percentage of that species collected at ground level (number collected at ground level by either human collectors or dry ice-baited CDC-type light traps divided by total of that species collected at all elevations).

<sup>e</sup> Percentage of that species collected at night (number collected at night by either human collectors or dry ice-baited CDC-type light traps divided by total of that species collected at all time periods).

<sup>f</sup> *Cx. (Mel.) spp. (total), all Cx. (Melanoconion)* captured by all methods.

<sup>g</sup> *Cx. (Mel.) spp., all Cx. (Melanoconion)* captured other than those identified to species elsewhere in the table.

<sup>h</sup> *Cx. (Cux.) spp., all Cx. (Culex) species combined.* These consisted almost entirely of *Cx. coronator*, *Cx. declarator*, and *Cx. mollis*.

<sup>i</sup> *An. spp.*, combined data for all 10 species of *Anopheles* captured (see Table 1).

the 24-h cycle (Fig. 3). In general, three peak activity periods were observed (0600, 1200, and 1800 hours). Peak activity that was observed at 1200 hours was due almost exclusively to the increased activity of *Ps. albigena* (Fig. 3b). Human attraction activity was highest during 1800–1840 hours, when both nocturnal and diurnal species were active. A similar peak in mosquito activity was observed at dawn during the 0500–0540-hour collections. Activity frequencies were relatively low between 2400 and 0540 hours, and the lowest activity was observed at 0400–0440 hours, just before dawn. *Psorophora ferox* (Von Humboldt), *Ps. albigena*, *Oc. serratus*, *Sabettus* spp., and *Hemagogus* spp. were active primarily during the daytime, whereas *Cx. pedrooi*, *Cx. vomerifer/gnomatos*, *Mansonia* spp., *Coquillettidia* spp., and other *Culex* species were active primarily during darkness (Fig. 3b and c). *Oc. fulvus*, although more frequently collected during the evening, was collected throughout the 24-h cycle (Fig. 3b).

**Collections at Human versus Dry Ice-Baited CDC-Type Light Traps.** Mosquito species and the number of the same species attracted to human collectors differed from those captured in dry ice-baited LTs. Overall, 44% of the mosquitoes were captured in HLCs (Table 2). During the daytime (0600–1740 hours) more mosquitoes were collected in HLCs (62%) than

were captured in the dry ice-baited LTs. In contrast, during darkness, only 32% of the mosquitoes were collected in HLCs compared with 68% collected in the dry ice-baited LTs (Tables 3 and 4). Of the more frequently captured mosquitoes, three (*Ps. albigena*, *Ps. ferox*, and *Oc. serratus*) were collected more frequently during the daytime (0600–1800 hours), whereas *Cx. pedrooi*, *Cx. vomerifer/gnomatos*, *Cx. (Mel.) spp.*, and *Cx. (Cux.) spp.* were collected almost entirely during darkness (1800–0600 hours). *Oc. fulvus* was collected throughout the 24-h cycle but more frequently during the hours of darkness. Several species [*Johnbelkinia longipes* (F.), *Hemagogus janthinomys* Dyar, *Hemagogus baresi* Cerqueira, *Sabettus chloropterus* (von Humboldt), *Ochlerotatus argyrothorax* (Bonne-Wepster & Bonne), *Mansonia amazonensis* (Theobald), and *Mansonia humeralis* Dyar & Knab] were captured infrequently and almost exclusively (>90%) in HLCs (data not shown). Except for *Culex (Melanoconion) portesi* Senevet & Abonnenc and *Cx. (Mel.) ocoosa*, other members of *Culex (Mel.)* were more frequently collected in dry ice-baited LTs (data not shown).

**Canopy versus Ground.** Adult mosquito activity for selected species collected at ground level versus canopy is shown in Tables 2–4. Overall, 1.5 times as many mosquitoes were collected near the ground (0–0.5 m

**Table 3.** Mean numbers of mosquitoes captured in human-landing collections from September 1996 through October 1997 ( $n = 100$  trap days) in a forested area, near Puerto Almendras, Peru

Species	Day <sup>a</sup>		Night <sup>b</sup>		Total <sup>c</sup>
	Canopy <sup>e</sup>	Ground <sup>d</sup>	Canopy <sup>c</sup>	Ground <sup>d</sup>	
<i>An. spp.<sup>f</sup></i>	0.1	1.8	1.1	1.3	4.3
<i>Cq. (Ryp.) venezuelensis</i>	0.4	0.6	3.5	5.5	10.1
<i>Cx. (Cux.) spp.<sup>g</sup></i>	0.6	1.1	5.6	13.4	20.8
<i>Cx. (Mel.) Atratus Group</i>	0.0	0.4	0.1	0.6	1.1
<i>Cx. (Mel.) ocoesa</i>	0.5	0.2	1.8	7.6	10.1
<i>Cx. (Mel.) pedroi</i>	1.1	0.2	12.2	32.6	46.2
<i>Cx. (Mel.) spissipes</i>	0.1	0.0	0.7	2.3	3.1
<i>Cx. (Mel.) theobaldi</i>	<0.1	<0.1	<0.1	0.3	0.3
<i>Cx. (Mel.) vomerifer/gnomatos</i>	0.5	0.3	9.1	39.1	49.1
<i>Cx. (Mel.) spp.<sup>h</sup></i>	5.6	17.0	2.7	6.2	31.5
<i>Cx. (Mel.) spp. (totals)<sup>i</sup></i>	7.8	18.1	26.6	88.7	141.3
<i>Hg. (Hag.) janthinomys</i>	4.6	2.0	0.0	0.0	6.6
<i>Li. flavisetosus</i>	2.0	15.6	0.0	0.1	17.7
<i>Li. durhamii</i>	0.5	2.5	0.0	0.0	3.1
<i>Ma. (Man.) indubitans</i>	2.9	0.8	9.5	5.3	18.6
<i>Oc. (Och.) fulvus</i>	23.5	39.1	80.8	101.0	244.3
<i>Oc. (Och.) hastatus</i>	1.2	2.3	0.7	0.6	4.9
<i>Oc. (Och.) serratus</i>	6.4	65.8	6.7	26.9	105.9
<i>Ps. (Gra.) cingulata</i>	0.2	0.1	0.7	2.5	3.5
<i>Ps. (Jan.) albigena</i>	83.1	239.4	13.6	23.0	359.0
<i>Ps. (Jan.) ferox</i>	7.1	35.5	0.8	2.5	45.8
Other (36 species)	5.8	12.7	2.9	8.2	29.7
Totals for all species	146.2	437.6	152.2	275.0	1,011.1
Percentage of total	14.4	43.3	15.1	27.2	100

<sup>a</sup> Mosquitoes were collected from 0600 to 1740 hours.

<sup>b</sup> Mosquitoes were collected from 1800 to 0540 hours.

<sup>c</sup> Human collectors sat on a platform in the forest canopy (10 m above ground level) with a thatched roof 1.5 m above the platform to protect the collectors from the elements.

<sup>d</sup> Human collectors sat on a log 0.5 m above the forest floor.

<sup>e</sup> Mean number of that species collected per 24 h by both collectors combined.

<sup>f</sup> *An. spp.*, combined data for all 10 species of *Anopheles* captured (see Table 1).

<sup>g</sup> *Cx. (Cux.) spp.*, all *Cx. (Culex)* species combined. These consisted almost entirely of *Cx. coronator*, *Cx. declarator*, and *Cx. mollis*.

<sup>h</sup> *Cx. (Mel.) spp.*, all *Cx. (Melanoconion)* captured other than those identified to species elsewhere in the table.

<sup>i</sup> *Cx. (Mel.) spp. (total)*, all *Cx. (Melanoconion)* captured by all collectors.

humans; 1.5-m LTs) than in the canopy ( $\approx 10$  m above the ground) for both collection methods combined (Table 2). *Ps. albigena* were more frequently collected at ground level, and the midday peak in biting activity (landing collections) at ground level was due almost exclusively to high populations of this species. *Sabettus* spp., *Hg. janthinomys*, *Jb. longipes*, and *Ma. indubitans* were captured more frequently in HLCs in the canopy (data not shown), whereas all other species were more frequently collected at ground level (Table 2). Although several species [e.g., *Oc. fulvus* and *Coquillettidia venezuelensis* (Theobald)] were collected more frequently in HLCs than in LTs at ground level, they were collected more frequently in LTs than in HLCs in the canopy (Tables 3 and 4).

### Discussion

During this study, we collected at least 70 species of mosquitoes, representing 14 genera, from a sylvatic site near Iquitos, Peru. These data represent a markedly higher number of species than the 37 species from 13 genera collected in the same general area during the early 1990s (Need et al. 1993). One reason for the increase in the number of mosquito species captured was the additional taxonomic efforts, including larval rearings, which enabled separation of many of the

members of species complexes into distinct species. These data extend previous observations reported for a larger study of mosquitoes in the Amazon Basin region during which a total of 96 species were captured and identified in urban, suburban, and sylvatic areas around Iquitos (Pecor et al. 2000).

The taxonomy of many mosquito "species" is poorly understood, making it difficult to describe their distribution and abundance. In a preliminary study, only five species of *Culex (Mel.)* spp. could be identified to species. Subsequently, larval and pupal exuviae and males were collected, resulting in the identification and confirmation of *Cx. gnomatos* (previously included with *Cx. vomerifer*), *Culex (Melanoconion) dunni* Dyar, *Culex (Melanoconion) theobaldi* (Lutz), and others (Table 1) (Pecor et al. 2000; J.E.P., unpublished data). Similar taxonomic problems apply to *Culex (Cux.)* spp., *Sabettus* spp., and *Wyeomyia* spp. *Oc. serratus* consisted of two distinct morphological forms (Pecor et al. 2000) but are included here as a single species. However, preliminary studies and observations, including progeny rearing and seasonal activity, suggest they may be distinct species based on both morphological and behavioral differences (e.g., members of a progeny brood were of the same form as the parent and relative proportions of the two forms

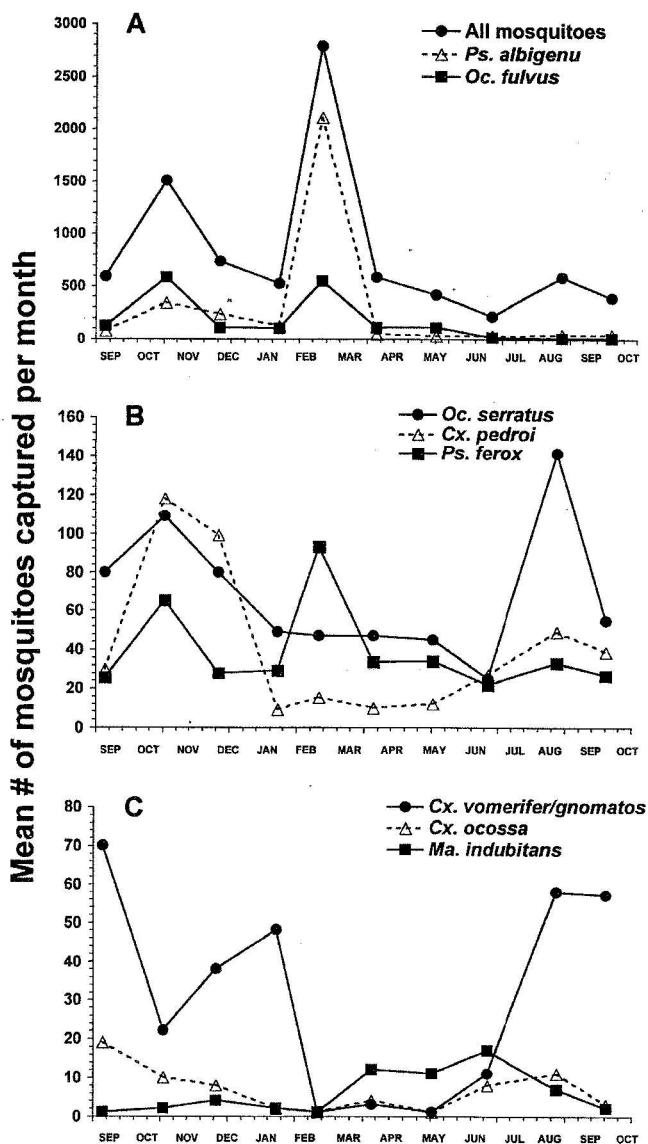


Fig. 2. Comparison of seasonal distribution of all mosquitoes and selected species captured by human collectors at ground level, Puerto Almendras Forest, September 1996–October 1997.

varied by season). As these problems are resolved, more accurate disease-threat analyses can be made.

The tropical and subtropical regions of South America range from semiarid high plateaus to coastal and inland rainforests. These extremes in environment and habitat provide for a wide range of species diversity and population dynamics among culicine mosquitoes that are potential vectors of arboviruses throughout the South American region. In most previous surveys conducted in South America, the species diversity was relatively low (range, 14–50 identified species) compared with the species diversity found in our study of a forested habitat in Peru. Guimaraes et al. (1989) reported 50 species of culicine mosquitoes from a National Park forest (Serra dos Orgaos) in Rio de Janeiro State, Brazil, of which only 10 species were collected at our study area near Puerto Almendras. In a field investigation in Belterra, Para, Brazil, Hoch et al. (1981) reported only 18 species of which only eight were collected at our study area. A malaria study conducted at Costa Marques, Rondonia, Brazil, ≈800 km from Iquitos, Peru, reported 14 species, 10 of which were collected in both areas (Klein et al. 1992). In a spatial dispersion study of mosquitoes in a sylvatic focus of VEE conducted in Venezuela, 34 species were reported, 20 of them were found in our study (Méndez et al. 2001). Some species (e.g., *Oc. serratus*, *Ps. ferox*, and *Ps. albigena*) are widespread and are frequently collected in HLCs throughout their range. Some of the under reporting of species may be due to the grouping

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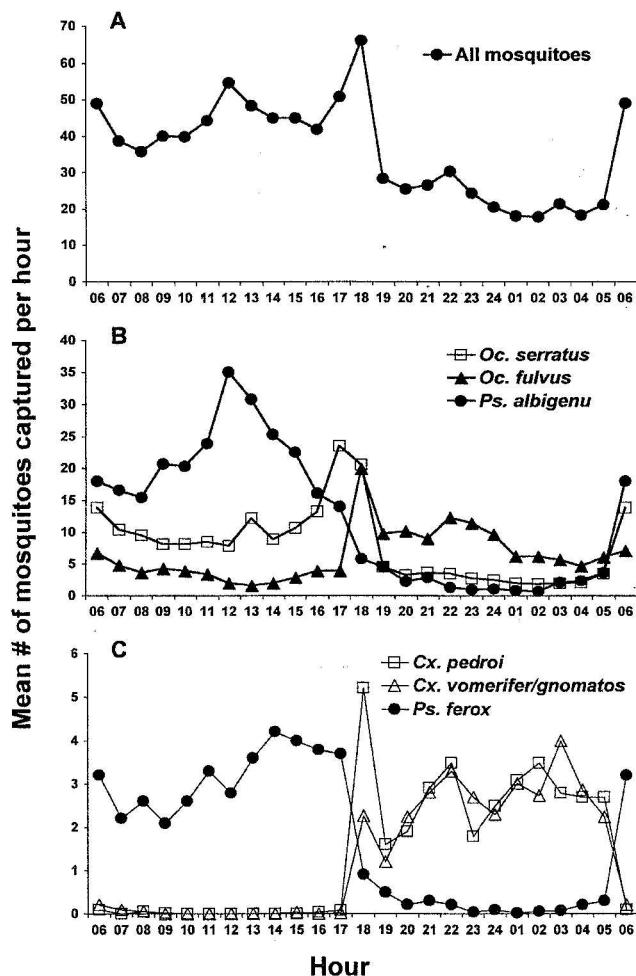


Fig. 3. Comparison of the mean number of selected species captured hourly by human collectors, Puerto Almendras Forest, September 1996–October 1997. Data are shown for collections made at the forest floor.

of mosquito species that could not be identified because of insufficient taxonomic information. Similarly, as reported in this study, many of the mosquitoes pooled for virus isolation were grouped as species groups [e.g., *Cx. (Mel.) Atratus Group*], or as subgenera [*Cx. (Cux.)* spp., *Cx. (Mel.)* spp.] or undescribed species (e.g., species 1), because females could not be distinguished from other species within a group or subgenus or had yet to be described.

Environmental factors (e.g., rainfall, flooding, and river level) are perhaps primary factors for changes in mosquito populations over time. During this study, the overall total culicine mosquito catches were highest from October through March (Fig. 2a). This corresponded to the rainy season, which normally commences in September and ends in May. This was especially true for "flood water" mosquitoes (*Oc. serratus*, *Oc. fulvus*, and *Ps. albigena*) that depend on sufficient rainfall for immersion hatching and development. The development from egg to adult of many of the floodwater mosquitoes (e.g., *Oc. serratus*) is

relatively short ( $\approx 7$ – $10$  d in the laboratory) (data not shown), resulting in rapid increases in populations. In our study, *Oc. fulvus* and *Oc. serratus* accounted for 22 and 6%, respectively, of the total number of mosquitoes captured throughout the collection period. In a similar study in Brazil (Ecological Station of Samuel), these same two species accounted for 25 and 10%, respectively, of the mosquitoes collected (Luz and Lourenco-de-Oliveira 1996). Although the flood water mosquito populations decreased during the dry season, others [e.g., *Mansonia titillans* (Walker) and *Ma. indubitanus*] rebounded during the dry season (Fig. 2c). Both *Ma. titillans* and *Ma. indubitanus* are very aggressive biters and have been incriminated in arbovirus transmission (Lourenco-de-Oliveira 1984, Lourenco-de-Oliveira and Heyden, 1986).

In Loreto Department, the Amazon River level fluctuates  $\approx 10$  m from a high in May to a low in October, even though an average of  $>200$  mm of rain is received monthly. Many of the mosquito species are dependent upon rain to fill small impoundments [e.g., leaf axils

**Table 4.** Mean numbers of mosquitoes captured in dry ice-baited CDC-type light traps from September 1996 through October 1997 ( $n = 100$  trap days) in a forested area near Puerto Almendras, Peru

Species	Day <sup>a</sup>		Night <sup>b</sup>		Total <sup>e</sup>
	Canopy <sup>c</sup>	Ground <sup>d</sup>	Canopy <sup>c</sup>	Ground <sup>d</sup>	
<i>An.</i> spp. <sup>f</sup>	0.1	0.2	8.9	2.7	11.9
<i>Cq. (Ryu.) venezuelensis</i>	<0.1	<0.1	4.7	2.3	7.0
<i>Cx. (Cux.)</i> spp. <sup>g</sup>	0.3	0.4	80.0	81.6	162.3
<i>Cx. (Mel.) Atratus Group</i>	<0.1	<0.1	2.8	3.3	6.1
<i>Cx. (Mel.) ocoosa</i>	<0.1	<0.1	1.5	5.4	6.9
<i>Cx. (Mel.) pedrooi</i>	0.1	<0.1	70.6	71.9	142.6
<i>Cx. (Mel.) spissipes</i>	<0.1	<0.1	3.4	10.5	13.9
<i>Cx. (Mel.) theobaldi</i>	0.7	<0.1	6.7	10.3	17.7
<i>Cx. (Mel.)</i> spp. <sup>h</sup>	2.6	4.5	88.1	87.4	182.6
<i>Cx. (Mel.)</i> spp. (totals) <sup>i</sup>	3.5	5.3	180.2	235.5	424.5
<i>Cx. (Mel.) vomerifer/gnomatos</i>	0.1	0.7	7.3	50.7	58.8
<i>Hg. (Hag.) janthinomys</i>	0.2	<0.1	<0.1	<0.1	0.2
<i>Li. (Lim.) flavisetosus</i>	0.8	8.4	<0.1	<0.1	9.2
<i>Li. (Lim.) durhami</i>	0.6	5.8	<0.1	<0.1	6.4
<i>Ma. (Man.) indubitans</i>	0.1	0.1	1.8	0.6	2.6
<i>Oc. (Och.) fulvus</i>	26.1	27.2	133.1	74.8	261.2
<i>Oc. (Och.) hastatus</i>	1.1	5.8	0.1	0.1	7.1
<i>Oc. (Och.) serratus</i>	1.2	11.8	7.5	10.9	31.4
<i>Ps. (Gra.) cingulata</i>	<0.1	<0.1	0.4	2.5	2.9
<i>Ps. (Jan.) albigena</i>	120.7	120.1	16.8	20.9	278.5
<i>Ps. (Jan.) ferox</i>	0.5	9.4	1.8	3.9	15.6
OTHER (36 species)	1.1	13.8	27.4	14.4	65.7
Totals for all species	156.3	208.2	462.9	454.2	1,281.6
Percentage of total	12.2	16.2	36.1	35.4	100

<sup>a</sup> Mosquitoes were collected from 0600 to 1800 hours.

<sup>b</sup> Mosquitoes were collected from 1800 to 0600 hours.

<sup>c</sup> Dry ice-baited CDC-type light traps were set in the forest canopy (10 m above ground level).

<sup>d</sup> Dry ice-baited CDC-type light traps were set 1.5 m above the forest floor.

<sup>e</sup> Mean number of mosquitoes collected per 24 h by both dry ice-baited CDC-type light traps combined.

<sup>f</sup> *An.* spp., combined data for all 10 species of *Anopheles* captured (see Table 1).

<sup>g</sup> *Cx. (Cux.)* spp., all *Cx. (Culex)* species combined. These consisted almost entirely of *Cx. coronator*, *Cx. declarator*, and *Cx. mollis*.

<sup>h</sup> *Cx. (Mel.)* spp., all *Cx. (Melanoconion)* captured other than those identified to species elsewhere in the table.

<sup>i</sup> *Cx. (Mel.)* spp. (total), all *Cx. (Melanoconion)* captured by all traps.

(*Wyeomyia* spp.), tree holes (*Sabettus* spp. and *Limatus* spp.), and streams (*Culex [Melanoconion] olímpioi* Xavier, Da Silva & Da Silva Mattos)]. Larval habitats of many of the species collected are poorly known. For example, our surveys of larval habitat at the forest site and the surrounding area failed to yield many of the species collected as adults (e.g., *Cx. pedrooi*, *Cx. vomerifer*, or *Cx. theobaldi*), even though large numbers of adults of these species were collected by LTs and in HLCs in the area surveyed. Although we made substantial progress in this study on the bionomics and ecology of mosquitoes, further studies are warranted to better understand the relative importance of mosquitoes as potential vectors of arboviruses and other pathogens in the Amazon Basin region. These would include virus isolation studies to determine which viruses are currently being transmitted in this region, vector competence studies to determine which of these mosquito species are able to transmit these viruses, and blood meal identification studies to determine on which vertebrate species these mosquitoes feed. Such information is critical for developing and implementing vector targeted control strategies.

The feeding patterns for mosquitoes observed in our study were similar to those reported by others. Klein et al. (1992) reported that *Ps. albigena* was one of the predominant forest mosquitoes and would readily blood feed on persons resting in the forest. As

we worked or walked through forest habitats during the daytime at our study site, these mosquitoes would follow and attempt to blood feed, especially when we stopped for a few minutes. Although *Ps. albigena* would infrequently follow collectors and workers more than several meters from the forest edge, they were collected near residences with nearby groups of trees (Klein et al. 1992; data not shown).

In our study, all *Culex (Mel.)* spp. accounted for 14% of all mosquitoes captured in HLCs. Members of *Culex*, subgenus *Melanoconion*, were frequently collected in the forest habitat and less frequently near human dwellings where land had been cleared (unpublished data). This corresponds to the findings of Klein et al. (1992) where *Cx. (Mel.)* spp. were infrequently collected near houses but were frequently collected near small streams and stagnant pools associated with tall grass, shrubs, trees, or a combination.

Our results are also consistent with those of Forattini (1986a,b), who found that *Oc. serratus* biting activity was bimodal and associated with crepuscular periods in the early morning and late afternoon and that *Ps. ferox* began feeding during the early morning, with activity rapidly declining during the early evening. Guimaraes and Victorio (1986) showed that *Trichoprosopon digitatum* (Rondani) fed on humans primarily during the day, with peak biting activity between 1600 and 1800 hours, and *Limatus durhami*

Theobald, also a day biter, had heaviest biting activity between 1000 and 1600 hours. *Sa. chloropterus* was only collected during the daytime. Although various day biters (e.g., *Sabethes* spp. and *Hemagogus* spp.) were infrequently collected by either HLCs or dry ice-baited LTs, they are important vectors of arboviruses affecting human health. Thus, in the Amazon Basin, there is a requirement for 24-h vigilance for personal protection against these biting insects and disease transmission.

The isolation of >100 distinct arboviruses (many pathogenic to humans) from arthropods collected in South America (Karabatsos 1985) emphasizes the need for epidemiological studies and surveillance of vectors, reservoirs, and pathogens (Miles 1960; Causey et al. 1963, 1964; Dixon et al. 1981; Hoch et al. 1981; Calisher et al. 1982; Pannuti et al. 1989; Momen 1998). Although few epidemics have been described, Dixon et al. (1981) found that a larger percentage of the local populations residing in the Amazon Basin were seropositive for arboviruses compared with rates in recent immigrant populations. Dixon et al. (1981) also suggested that arbovirus transmission was largely sylvatic because attack rates were higher for adult males who slept or worked in forested areas. The increasing exploitation of sylvatic resources, urbanization, and focal military operations (e.g., drug interdiction, border disputes, and humanitarian aid) in South America places civilians and military populations at risk as they come into direct contact with vectors and the viruses they transmit. However, because of the lack of funding and logistical support, inadequate or nonexistent local medical facilities, unavailable diagnostics, and lack of knowledge of the epidemiology and distribution of arboviruses, the etiology of fevers of unknown origin and even deaths often are undiagnosed or are misattributed to other more common diseases. The resurgence of malaria and introduction of dengue into urban areas has greatly increased morbidity and mortality among civilian populations. Therefore, the principal emphasis of the vector-borne disease programs of the Peruvian Ministry of Health, Loreto Department, Peru, has been focused on the control of malaria and dengue vectors. However, more attention needs to be given to culicine mosquitoes and other biting Diptera that transmit a variety of other parasites and arboviruses (e.g., *Leishmania* spp., *Onchocerca* spp., and numerous viruses, including VEE, eastern equine encephalomyelitis, and various members of the genus *Bunyavirus*) that affect animal and human health in the Amazon Basin (Klein et al. 1992, Luz and Lourenco-de-Oliveira 1996, da Silva 1998). Most patients are seen only after they have failed to respond to treatment for bacterial and protozoan infections. At this time, it is too late for virus isolation, even though the illness may be one of arbovirus etiology (Causey et al. 1961). Although testing acute and convalescent sera from surviving patients may assist in identifying the causative agent, this is limited because of the unavailability of serological tests needed to identify pathogens that are generally known to circulate in the region.

Global surveillance serves as an early warning system for epidemics and provides a rationale for public health interventions (Broome 1998). Concerns about communicable disease in both industrialized and developing countries can best be addressed through strong surveillance systems, renewed commitment to public health, and strong international partnerships to strengthen national and international cooperation in communicable disease prevention and control (Broome 1998). However, to date, the most reliable method for determining risk of human infection is to capture mosquitoes as they come to feed on human collectors (e.g., landing collections and Shannon traps). Even though mosquitoes are collected after landing on collectors, before they feed, if at all possible, a few may probe and take partial blood meals before being aspirated, exposing the collectors to the risk of arboviral infections, as well as to infections with other pathogens (e.g., malaria, filaria, and leishmania). Although the use of dry ice-baited LTs may reduce the risk of human infection, they may not capture some species of mosquitoes that are principal vectors of selected arboviruses, e.g., *Sabethes* spp. and *Hemagogus* spp. Also, the number of mosquitoes collected in various traps has not been correlated with human exposure, and trap attraction versus human attraction varies with each species and population density. For example, *Hg. janthinomys* (Dyar), the principal vector of Mayaro in some areas of Brazil, is almost exclusively collected in HLCs or animal-baited trap collections compared with dry ice-baited LTs. In contrast, other species are predominately collected in LTs [e.g., most *Cx. (Mel.)* spp.] (Hoch et al. 1981). Also, without an attractant (e.g., dry ice or CO<sub>2</sub>), which may be difficult to obtain in remote areas, LTs are relatively inefficient for most species, and in particular those that are active during the daytime. Tables 2–4 identify some of the differences in HLCs versus dry ice-baited LTs. This information can be used to predict the number expected to be collected in HLCs based on the numbers observed at dry ice-baited LTs. New and novel surveillance tools need to be developed that provide real-time information on vector abundance and risk of virus transmission. For example, in Korea, Burkett et al. (2001) showed that LTs baited with CO<sub>2</sub> were ≈10 times more effective than unbaited traps, whereas new traps with counterflow technology (e.g., Mosquito Magnet) were >100 times more effective. Although correlations of trap collections versus human attraction have not been established, using more efficient trapping methods provides increased efficiency for epidemiological studies requiring large numbers of mosquitoes for arbovirus assay.

The importance of early identification of arboviruses and the role of primary diagnostic laboratories and the reference laboratory networks that support them must also be recognized. In this era of the primacy of molecular microbiology and virology, we should remember that many of the early investigative activities surrounding the identification of potential emergent zoonotic diseases must be carried out in the field, and not in the laboratory. Our study of mosquito

bionomics is part of a larger study that includes surveys of mosquitoes for emerging arboviral pathogens, vector competence for selected viruses, seroprevalence, and disease incidence studies (reported separately). Together, these studies will provide a better understanding of vector populations, distribution of arboviruses, and disease risks within the Amazon Basin region and allow for the development and implementation of appropriate prevention strategies that can be developed for urban and rural communities, as well as various types of military operations and training.

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